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Catalytic Reactions in a Polymeric Model System for Hydrogen Peroxide Delignification of Pulp

C.C. Walker, T.J. McDonough, R.J. Dinus, and K.-E.L. Eriksson

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Catalytic Reactions in a Polymeric Model System for Hydrogen Peroxide Delignification of Pulp

Colleen C. Walker, Graduate Student
Thomas J. McDonough, Professor of Engineering
Ronald. J. Dinus, Professor of Biology
Institute of Paper Science and Technology
500 10th Street, N.W.
Atlanta, GA 30318

Karl.-Erik L. Eriksson
Professor of Biochemistry
Life Sciences Building
University of Georgia
Athens, GA 30602

ABSTRACT

A unique experimental system has been developed to study the effect of three iron-based catalysts on the hydrogen peroxide oxidation of polymeric models of lignin and carbohydrate. Iron chelated by sequestering agents or bound in porphyrin structures, in the presence of hydrogen peroxide or other oxidants, mimics the delignifying action of lignin peroxidase. The selectivity of these so-called biomimetic compounds for lignin over carbohydrate is the subject of this study.

Lignosulfonate and hydroxyethyl cellulose (HEC) have been chosen as the polymeric models for lignin and carbohydrate. Molecular weights of these substrates were measured by High Performance Size-Exclusion Chromatography (HPSEC) and viscometry, respectively. Reactions were performed at pH 3.0, the optimum for lignin peroxidase activity. The three catalysts evaluated were $FeSO_4$, Fe-EDTA, and hemoglobin.

Rates of lignosulfonate and hydroxyethyl cellulose degradation were separately determined in the presence of each catalyst. The two were then compared to determine the selectivity of each catalytic system. In experiments in which both lignosulfonate and HEC were combined in reaction solutions, the formation of a large molecular weight product was observed. This product is presumably the result of a condensation reaction between the lignin and cellulose models. Its formation is significant inasmuch as it models a counterproductive process that may be responsible for the limited effectiveness of enzymatic delignification systems.

INTRODUCTION

Environmental concerns have forced the pulp and paper industry to examine alternatives to the bleaching of pulp with chlorine and chlorine-containing compounds. Delignification with oxygen-containing agents, such as oxygen, hydrogen peroxide and ozone, has necessarily become a more attractive bleaching technology. Biotechnological applications are also receiving increased attention as viable alternatives for traditional chlorine bleaching (Yang et al. 1992).

The isolation and subsequent characterization of enzymes which are capable of degrading wood components have created numerous opportunities for applications of biotechnology to the production of pulp and paper. Lignin peroxidase, isolated from the white-rot fungus Phanerochaete chrysosporium, is a lignin-degrading enzyme which has been widely studied (Eriksson et al. 1990; Higuchi 1985). This enzyme possesses an iron protoporphyrin IX prosthetic group, the nonamino acid portion of the enzyme, and is dependent on hydrogen peroxide for activity (Tien and Kirk 1983; Glenn et al. 1983).

Researchers have used various iron complexes to mimic the heme group of lignin peroxidase, both to obtain a better understanding of the enzymatic mechanisms involved in lignin biodegradation, and to investigate their potential as bleaching agents. Combinations of these so-called biomimetic compounds with hydrogen peroxide and other oxidants have been applied to lignin model compounds (Shimada et al. 1989; Huynh 1986), wood pulp (Pettersson et al. 1988; Crawford and Paszczynski 1989; Paszczynski et al. 1988; Skerker et al. 1989) and chips (Paszczynski et al. 1988).

For biomimetic bleaching systems to be considered commercially feasible, they must demonstrate a selectivity which favors lignin removal. Hydroxyl radicals have been observed in solutions containing both hydrogen peroxide and iron (Walling 1975) or iron complexes (Sepp et al. 1992; Puppo and Halliwell 1988). It is well known that $FeSO_4$ and hydrogen peroxide, more commonly known as Fenton's reagent, readily produce hydroxyl radicals (Walling 1975). The hydroxyl radical is known for its high reactivity and lack of selectivity. Evidence exists, however, that the hydroxyl radical reacts more rapidly with aromatic structures than with carbohydrates (Ek et al. 1989). The hydroxyl radical is believed to be a key radical species involved in alkaline hydrogen peroxide bleaching (Smith and McDonough 1985; Hobbs and Abbot 1991). Similar evidence is inconclusive for hydrogen peroxide delignification of pulp under acidic conditions (Hobbs and Abbot 1991).

The objective of this work was to evaluate the selectivity of three different types of biomimetic compounds. Soluble polymeric models for lignin and carbohydrate were selected to provide a homogeneous, yet realistic reaction system. Depolymerization rates were determined for each substrate separately and then compared. Preliminary results from experiments in which both substrates were combined in reaction solutions are briefly discussed.

EXPERIMENTAL APPROACH

Three different types of biomimetic, iron-based catalysts were chosen for evaluation: $FeSO_4$, Fe-EDTA, and hemoglobin. $FeSO_4$ represents the simplest form of a biomimetic

compound, and is known to generate hydroxyl radicals if combined with hydrogen peroxide under the right conditions. Fe-EDTA represents biomimetic compounds in which iron is strongly sequestered, in this case by EDTA (Bell 1977). The third model catalyst, hemoglobin, perhaps most resembles the enzyme lignin peroxidase, since its iron is surrounded by a complex matrix of protein.

Homogeneous reaction conditions in aqueous solution were desired; therefore, water-soluble model compounds representing wood pulp as closely as possible were considered. Lignosulfonate was chosen as a residual lignin model compound; hydroxyethyl cellulose (HEC) was chosen as a model for pulp carbohydrates. Changes in lignosulfonate molecular weight were measured using High Performance Size-Exclusion Chromatography (HPSEC). Changes in HEC molecular weight were measured by viscometry.

The reactor system used for these experiments was designed to measure the amount of hydrogen peroxide consumed in the chemical reactions as well as that which is catalytically decomposed to oxygen. Evolved oxygen was collected in an expandable column, which allows atmospheric pressure to be maintained in the reactor. By measuring the amount of oxygen evolved, the amount of hydrogen peroxide decomposed to oxygen was determined. Residual hydrogen peroxide was determined by titration. The amount of hydrogen peroxide consumed in the chemical reaction was then considered to be the difference.

Reaction conditions for experiments discussed in this paper are listed in Table 1. Concentrations of substrates and reactants were selected to resemble those commonly found in typical bleaching applications, while remaining within the constraints dictated by the analytical techniques. A pH of 3.0 was selected as it is that which the enzyme lignin peroxidase exhibits maximum activity (Aitken and Irvine 1989).

Table 1. Reaction conditions for catalyzed hydrogen peroxide oxidation of lignosulfonate and hydroxyethyl cellulose (HEC). All reactions were carried out at pH 3.0 and 45°C.

			CATALYST	
SUBSTRATE	**SUBSTRATE CONC. (g/l)**	**H_2O_2 (mM)**	**TYPE**	**CONC. (mM)**
Lignosulfonate	3.4	50.0	$FeSO_4$ Fe-EDTA Hemoglobin	0.5 0.5 0.062 (Fe)
Hydroxyethyl Cellulose	3.0	20.0	$FeSO_4$ Fe-EDTA Hemoglobin	0.2 0.2 0.025 (Fe)

The molar concentration of iron in hemoglobin was estimated from its structure. Hemoglobin has a molecular weight of 64,500 units with each molecule possessing four iron atoms. An extremely large concentration of hemoglobin (8.1 g/l) would be needed to match the 0.5 mM iron concentration used in the $FeSO_4$ and Fe-EDTA experiments. Consequently, the concentration of hemoglobin used in these experiments was arbitrarily selected.

RESULTS AND DISCUSSION

Catalyzed Oxidation of Lignosulfonate

The weight-average molecular weight of lignosulfonate versus reaction time for each catalyst is shown in Figure 1. Results from a control reaction, in which no catalyst was present, are also shown. All catalysts promote significant degradation of lignosulfonate as compared to the control. $FeSO_4$ causes the largest decrease in the molecular weight of lignosulfonate within the 400-minute reaction time.

Although not clearly visible in Figure 1, when hemoglobin is added to solutions containing lignosulfonate, prior to the addition of hydrogen peroxide, a reduction in the weight average molecular weight of lignosulfonate is observed (a decrease of approximately 1000 units). This gives the appearance of a larger overall reduction in lignosulfonate molecular weight for this reaction. Control experiments with hemoglobin and lignosulfonate, in the absence of hydrogen peroxide, showed that no significant degradation of the lignosulfonate occurred after this initial drop.

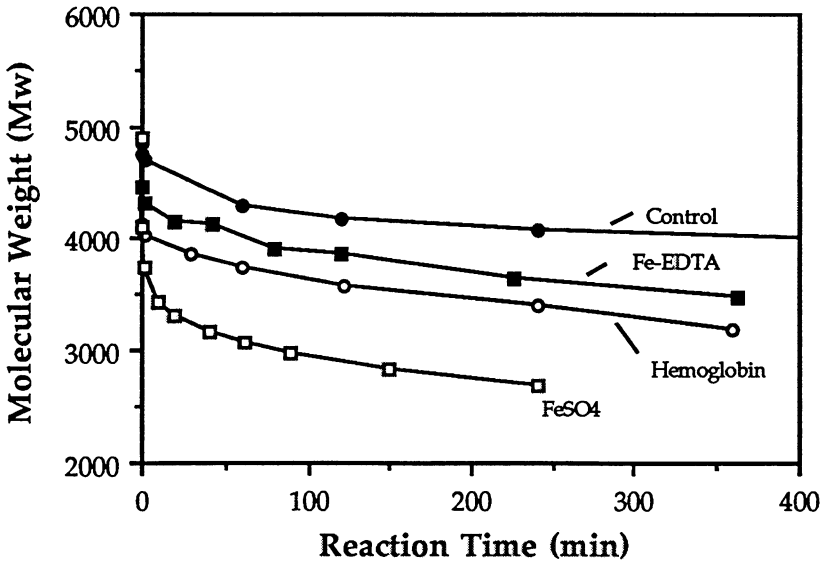

Figure 1. Weight-average molecular weights of lignosulfonate versus reaction time for each catalyst and a control, with H_2O_2 and no catalyst. See Table 1 for reaction conditions.

This initial decrease in molecular weight was not observed in either the $FeSO_4$ or Fe-EDTA catalyzed reactions. Similar control experiments with lignosulfonate and $FeSO_4$ or Fe-EDTA in the absence of hydrogen peroxide showed no degradation of the lignosulfonate.

Two possible explanations can be presented for this apparent decrease in molecular weight. In the presence of hemoglobin, the natural conformation of the lignosulfonate may be affected. As lignosulfonate then passes through the size-exclusion column, it appears to have a lower molecular weight. Alternatively, the hemoglobin may possess enough oxidizing potential to break only certain weak bonds in the lignosulfonate polymer, resulting in a decrease in the molecular weight. Efforts are continuing to determine the reason for this apparent decrease in molecular weight.

Corresponding changes in residual hydrogen peroxide concentration for the reactions shown in Figure 1 are shown in Figure 2. Here, with regard to the reaction of hydrogen peroxide, the addition of $FeSO_4$ results in the largest apparent consumption of hydrogen peroxide. Fe-EDTA and hemoglobin both catalyze the disappearance of hydrogen peroxide, but not to the same extent as $FeSO_4$.

The ratio of the amount of hydrogen peroxide decomposed to that consumed by reaction is plotted against the total amount of hydrogen peroxide reacted in Figure 3. The data for the control run shown in Figure 3 lack precision because oxygen evolution is minimal during the control run and little hydrogen peroxide reacts. However, it can be concluded that most of the hydrogen peroxide that disappeared in the control run was lost by conversion to oxygen.

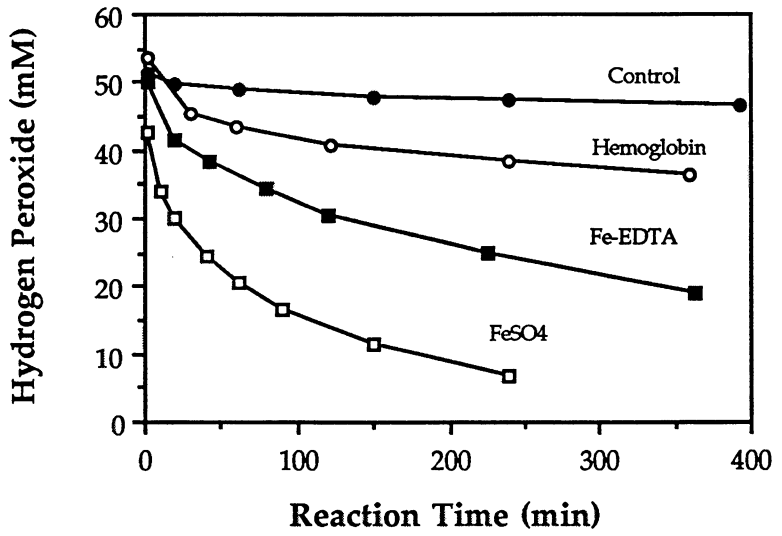

Figure 2. Residual hydrogen peroxide concentration versus reaction time for those experiments shown in Figure 1. See Table 1 for reaction conditions

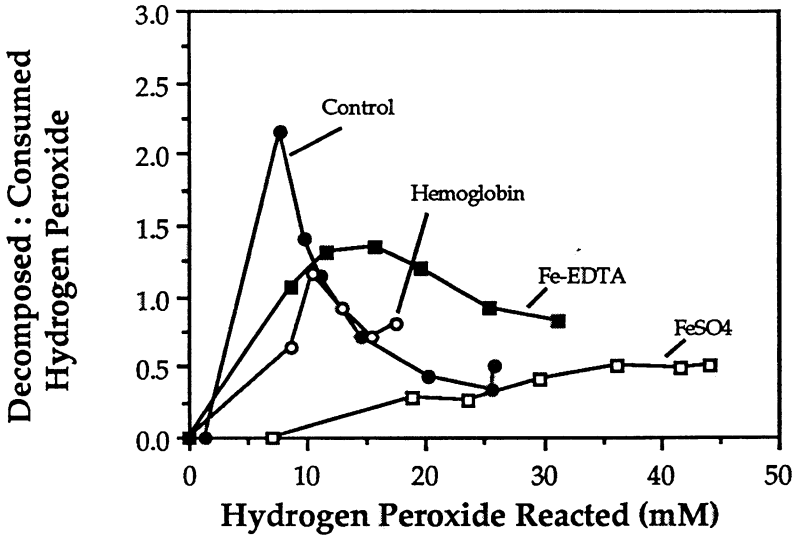

Figure 3. Ratio of hydrogen peroxide decomposed (to oxygen) to hydrogen peroxide consumed (by oxidation of lignosulfonate) versus total hydrogen peroxide reacted for those experiments shown in Figure 1.

$FeSO_4$ utilizes reacted hydrogen peroxide better than Fe-EDTA or hemoglobin. At a given amount of hydrogen peroxide reacted, for instance 20 mM H_2O_2, the ratio of decomposed to consumed hydrogen peroxide is significantly less than 1.0 for $FeSO_4$, indicating that most of the hydrogen peroxide has been consumed in the reaction. For Fe-EDTA and hemoglobin, this ratio is near 1.0, indicating that equal amounts of hydrogen peroxide are consumed and decomposed.

Kinetics of Lignosulfonate Depolymerization

The lignosulfonate polymer can be broken either by random chain scission or nonrandom processes such as bond-splitting at weak points in the polymer. If the process is random, the rate at which bonds are broken would not change with time. It can be shown that the number of chain scissions, N, can be determined from the degree of polymerization, DP, by:

$$N = \frac{1}{DP_t} - \frac{1}{DP_o} \qquad (1)$$

As DP is proportional to the weight-average molecular weight, Mw, Equation 1 can be rewritten in terms of Mw to give:

$$N = \frac{1}{Mw_t} - \frac{1}{Mw_o} \qquad (2)$$

The number of chain scissions plotted versus reaction time for all three catalysts and the control is shown in Figure 4. A straight line on this plot indicates that the rate of chain scission is constant throughout the reaction. It is apparent that this rate for the ferrous sulfate catalyzed oxidation of lignosulfonate varies greatly with time. For the Fe-EDTA and hemoglobin catalyzed reactions, the rate of chain scission decreases only slightly with respect to reaction time. The control experiment also shows a slight decrease in the rate of chain scission with reaction time.

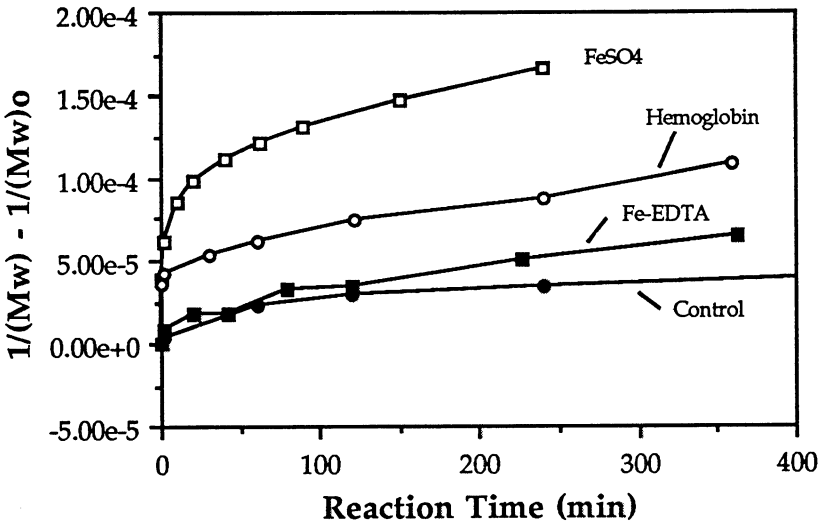

Figure 4. Number of chain scissions versus reaction time for those experiments shown in Figure 1.

In general, the rate of chain scission is expected to be dependent on the number of bonds present and the concentration of hydrogen peroxide. The rate equation applicable to all four reactions is therefore:

$$-\frac{d\,[\text{bonds}\}}{dt} = k_1\,[\text{bonds}]^a[\,C_H]^b$$

where $\mathbf{k_1}$ represents the overall rate constant, **a** the reaction order in bonds, and **b** the reaction order in hydrogen peroxide. For these systems, it is most likely that bonds are broken randomly, independent of the total concentration of bonds present. Therefore, the rate of chain scission is zero order in bonds. The rate equation then simplifies to:

$$-\frac{d\,[\text{bonds}\}}{dt} = k_1\,[\,C_H]^b \qquad (3)$$

The differential method was used to evaluate the reaction order **b** for the dependence of the rate on the hydrogen peroxide concentration. Reaction rates were determined from [bonds] versus time plots, where [bonds] = 1 - (1/Mw). Once the reaction order **b** was determined, the rate constant **k1** was calculated. Values of all parameters obtained for each catalyst are summarized in Table 2. For all reactions, the data fit the derived equations very well.

Table 2. Rate laws for the catalyzed hydrogen peroxide depolymerization of lignosulfonate.

CATALYST	H_2O_2 RANGE	RATE LAW
Control	50 - 30 mM	$r = 2.50 \times 10^{-10} [H_2O_2]^{1.3}$
$FeSO_4$	$[H_2O_2]$ 50 - 21 mM	$r = 2.35 \times 10^{-11} [H_2O_2]^{3.1}$
	$[H_2O_2] < 21$ mM	$r = 3.39 \times 10^{-11} [H_2O_2]^{0.86}$
Fe-EDTA	50 - 20 mM	$r = 2.10 \times 10^{-11} [H_2O_2]^{2.6}$
Hemoglobin	50 - 20 mM	$r = 7.90 \times 10^{-12} [H_2O_2]^{2.7}$

Catalyzed Oxidation of Hydroxyethyl Cellulose

Table 1 lists the reaction conditions used for these experiments. Viscosity-average molecular weights were determined using the Mark-Houwink equation, which relates intrinsic viscosity to molecular weight. Figure 5 shows the changes in viscosity-average molecular weight of HEC during the three catalyzed reactions and a control.

The $FeSO_4$ catalyzed reaction resulted in the greatest degradation of HEC. Fe-EDTA provides a sufficient catalytic environment to also degrade the HEC polymer. Hemoglobin, even at its relatively low molar iron concentration, also catalyzes the degradation of the HEC polymer.

For these experiments, a small change in the hydrogen peroxide concentration (< 20%) was observed during all reactions. It was therefore difficult to obtain information on the relative amounts of hydrogen peroxide which was consumed by reaction versus that decomposed to oxygen.

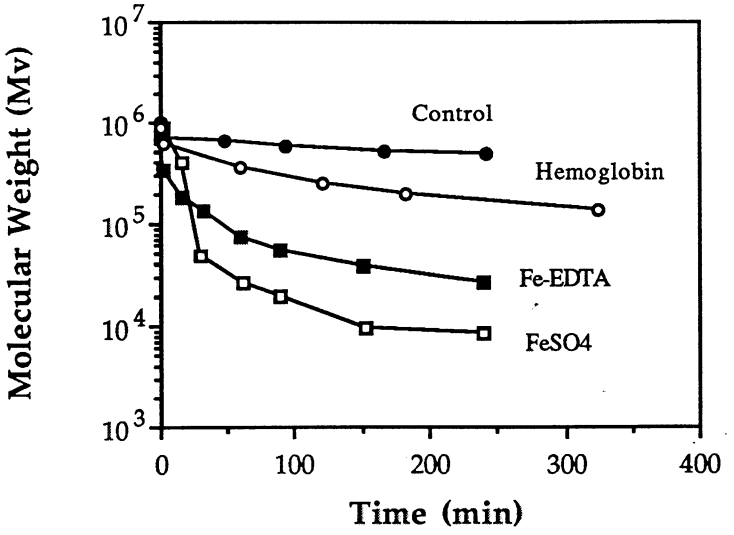

Figure 5. Viscosity average molecular weight versus reaction time for each catalyst and a control with H_2O_2 but no catalyst. See Table 1 for reaction conditions.

Kinetics of HEC Depolymerization

A plot of the number of chain scissions (Equation 2) during the reaction versus reaction time is shown in Figure 6 for each catalyst as well as the control (no catalyst). The straight lines in Figure 6 indicate that the depolymerization of HEC occurs at a constant rate throughout the reaction.

The generalized rate equation given above (Equation 3) can also be applied to the data from these experiments. For the depolymerization of HEC, the rate of chain scission is zero order in bonds, as shown in Figure 6. As mentioned previously, the hydrogen peroxide concentration remains relatively constant during these reactions. Consequently, a first-order dependence on the hydrogen peroxide concentration has been assumed. Experiments are being performed to verify this assumption. Table II lists values for k_1 determined from slopes in Figure 6.

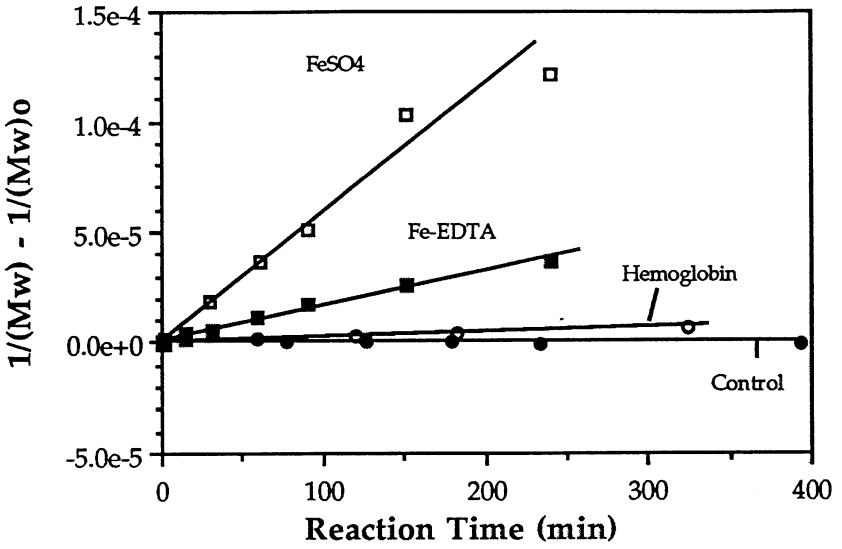

Figure 6. Number of chain scissions versus reaction time for each catalyst and a control (no catalyst). See Table 1 for reaction conditions.

TABLE 3. Rate constants for the catalyzed hydrogen peroxide depolymerization of HEC. Reaction conditions as given in Table 1.

CATALYST	RATE CONSTANT, k_1 (mM^{-1} min^{-1})
Control	0.15×10^{-9}
$FeSO_4$	27.50×10^{-9}
Fe-EDTA	7.70×10^{-9}
Hemoglobin	0.94×10^{-9}

Selectivity

Selectivity is most appropriately evaluated by comparing rate constants for the involved reactions. For the work presented here, a simple comparison of rate constants is meaningless since the rate laws for each catalyst in the lignosulfonate and HEC system are not identical. However, a point selectivity can be calculated and compared. A point selectivity is defined as the ratio of the two rates of interest evaluated at a certain point in the reaction.

Point selectivities were determined at a concentration of 20 mM hydrogen peroxide, as HEC reactions were performed at 20 mM. Rates of lignosulfonate depolymerization were calculated from the determined rate laws (Table 2), substituting a value of 20 mM for the hydrogen peroxide concentration. Results from this comparison are shown in Table 4. These results indicate that the ratio of the rate of lignosulfonate degradation to that of HEC degradation at 20 mM H_2O_2 is greatest in the case when hemoglobin is the catalyst. Fe-EDTA is next in its ability to degrade lignosulfonate more rapidly than HEC. $FeSO_4$ exhibits the least ability to degrade lignosulfonate over HEC.

Table 4. Point selectivities for the rate of bond cleavage in lignosulfonate to HEC determined at 20 mM H_2O_2.

CATALYST	POINT SELECTIVITY
$FeSO_4$	0.02
Fe-EDTA	6.49
Hemoglobin	26.6

These results may reflect the ability of these catalysts to generate radical species. The efficiency of hydrogen peroxide utilization must also be considered. The molecules or "cages" surrounding the iron may also contribute to the ability of the catalysts to associate with either substrate.

Combined Substrate Experiments

To provide a realistic reaction environment, lignosulfonate and HEC were combined in reaction solutions. The effect on reaction rates of the same three catalysts was evaluated. These results will be briefly described, with a more detailed description and discussion to be presented in a subsequent publication.

In all reactions, including that with hydrogen peroxide alone (no catalyst), analysis of reaction samples by HPSEC indicated the formation of a large molecular weight product. This product is believed to be produced by condensation of the lignosulfonate onto the HEC polymer.

Significant experimental evidence exists to support this hypothesis. This large molecular weight product was not observed to form in reactions with lignosulfonate in the absence of HEC.

Figure 7 shows chromatograms from several samples taken during the $FeSO_4$ catalyzed reaction. The molecular weight distribution of this condensation product changes during the reaction, shifting toward lower molecular weights, indicating that degradation has occurred.

For the $FeSO_4$ catalyzed reaction, the condensation product appears immediately (within 1 minute of reaction initiation). In the presence of Fe-EDTA, the condensation product appears a little more slowly, with full condensation of the lignosulfonate occurring after 10 minutes. In the presence of hemoglobin, only a small portion of the lignosulfonate condenses onto the HEC polymer.

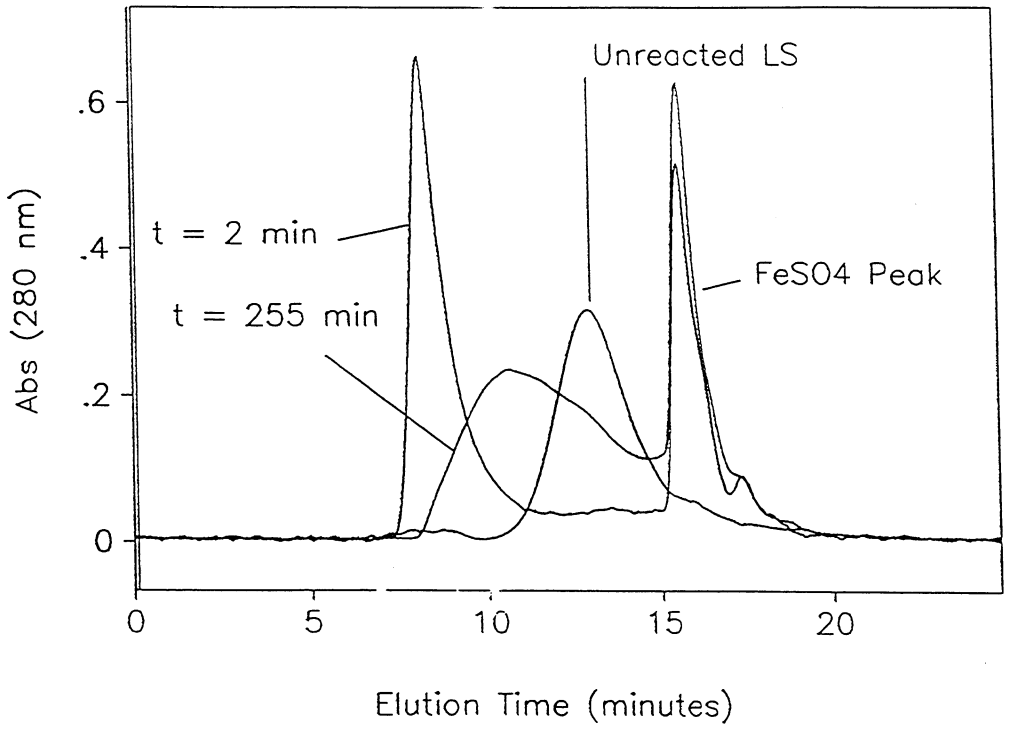

Figure 7. Size-exclusion chromatograms (UV Detector at 280 nm) from several reaction samples showing the large molecular weight product and the change in molecular weight distribution during reaction time. Reaction conditions: 3.0 g/l HEC, 0.5 g/l Lignosulfonate, 20 mM H_2O_2, 0.2 mM $FeSO_4$, pH 3.0, and 45°C.

CONCLUSIONS

Rate laws for the catalyzed hydrogen peroxide oxidation of lignosulfonate by three iron-containing catalysts were determined. Although the dependence on the hydrogen peroxide concentration differs for each catalyst, these values are relatively close together in magnitude. Rate laws were also determined for the hydrogen peroxide oxidation of HEC by the same three

catalysts. For these reactions, it was necessary to assume a first order dependence on hydrogen peroxide concentration, which is presently under investigation.

Point selectivities were determined from these rate equations at a concentration of 20 mM hydrogen peroxide. The ratio of the rate of chain scission in lignosulfonate to that of HEC was evaluated for each catalyst. These results indicate that $FeSO_4$ exhibits the highest selectivity at the chosen concentration. $FeSO_4$ is followed by Fe-EDTA and then hemoglobin.

In combined substrate experiments, the formation of a large molecular weight product was observed. This may provide new insight into the efficiency of hydrogen peroxide delignification of pulp. It is possible that the generation of hydroxyl radicals, and other active species, causes a polymerization to occur. This limits the efficiency of the bleaching agent to remove lignin from the pulp without also removing carbohydrate. Experimental conditions in which the formation of this condensation product is minimized may produce a more efficient bleaching agent.

EXPERIMENTAL METHODS

Chemicals and Substrates

Chemicals and Water

Ultrapure water was obtained from a Barnestead Reverse Osmosis Water Purifier configured to provide Class Type II lab water. Hydrogen peroxide (J. T. Baker) and hydrochloric acid were also of ultrapure quality. Hemoglobin (Sigma Chem. Co.) was bovine hemoglobin, unpurified. All other chemicals were of reagent grade.

Lignosulfonate

Lignosulfonate at ~ 50% solids was obtained from Daishowa Chem. Inc., Rothschild, Wisconsin. This solution was diluted, passed through a 0.22 μm Whatman filter, and then ultrafiltered using 30,000 and 10,000 daltons filters to obtain a fraction of relatively narrow molecular weight. Concentrations of stock solutions were determined by spectrometry; a standard calibration was made by using a solution whose solids content had been determined.

Hydroxyethyl Cellulose (HEC)

Hydroxyethyl cellulose was obtained from Aqualon Chem. Co., Wilmington, Delaware. Solutions were prepared by dissolving a preweighed amount of HEC into a known quantity of ultrapure water (usually 300 ml). Solutions were allowed to mix for 1 hour at room temperature and then overnight at 4°C to ensure complete swelling of the substituted cellulose.

Reactor and Reaction Sampling

All experiments were performed in a 300 ml teflon-lined, magnetically stirred batch reactor. The reactor was equipped with an air-tight lid, a sampling line, pH probe, and gas exit line. Samples were periodically removed and immediately placed on ice. Once cooled, aliquots were removed for titration, chromatography, or viscometry measurements.

High Performance Size-Exclusion Chromatography

A Bio-Rad Bio-Sil SEC 125 Size-Exclusion Column was used on a Varian 5060 High Performance Liquid Chromatograph equipped with a Varian UV-50 Variable Wavelength Detector and a Hewlett-Packard Integrator. The mobile phase (eluent) was 50 mM citric acid-disodium hydrogen phosphate buffer (Lewis and Yean 1985), pH 3.0, and flowed at 0.8 ml/min.

Viscometry

An existing correlation between intrinsic viscosity and molecular weight (the Mark-Houwink equation) was used to obtain molecular weight values. Mark-Houwink parameters for dilute HEC solutions were obtained from Brown et al. (1963). Samples were introduced into the viscometer (usually 1.0 ml) via pipet and allowed to equilibrate for 5 minutes (hydrogen peroxide was removed by addition of sodium bisulfite). Water was then added to reach the minimum volume necessary for measurement. Flow time was recorded for two passes; then, additional water was added.

ACKNOWLEDGMENTS

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